Azadinium isolation from sediment samples

Joo-Hwan Kim, Brian Bill, Nicolaus Adams and Vera L. Trainer

Hanyang University, Seoul, South Korea
NOAA-Fisheries Science Center, Seattle, WA, USA

Background

- Sediment incubation method has been u sed to isolate *Azadinium.*
- According to Adams, cell concentration of *Azdinium* spp. in Puget Sound is low (< 200 cells/L).
- Low cyst abundance of *Azadinium* spp. i s likewise expected in Puget Sound area.

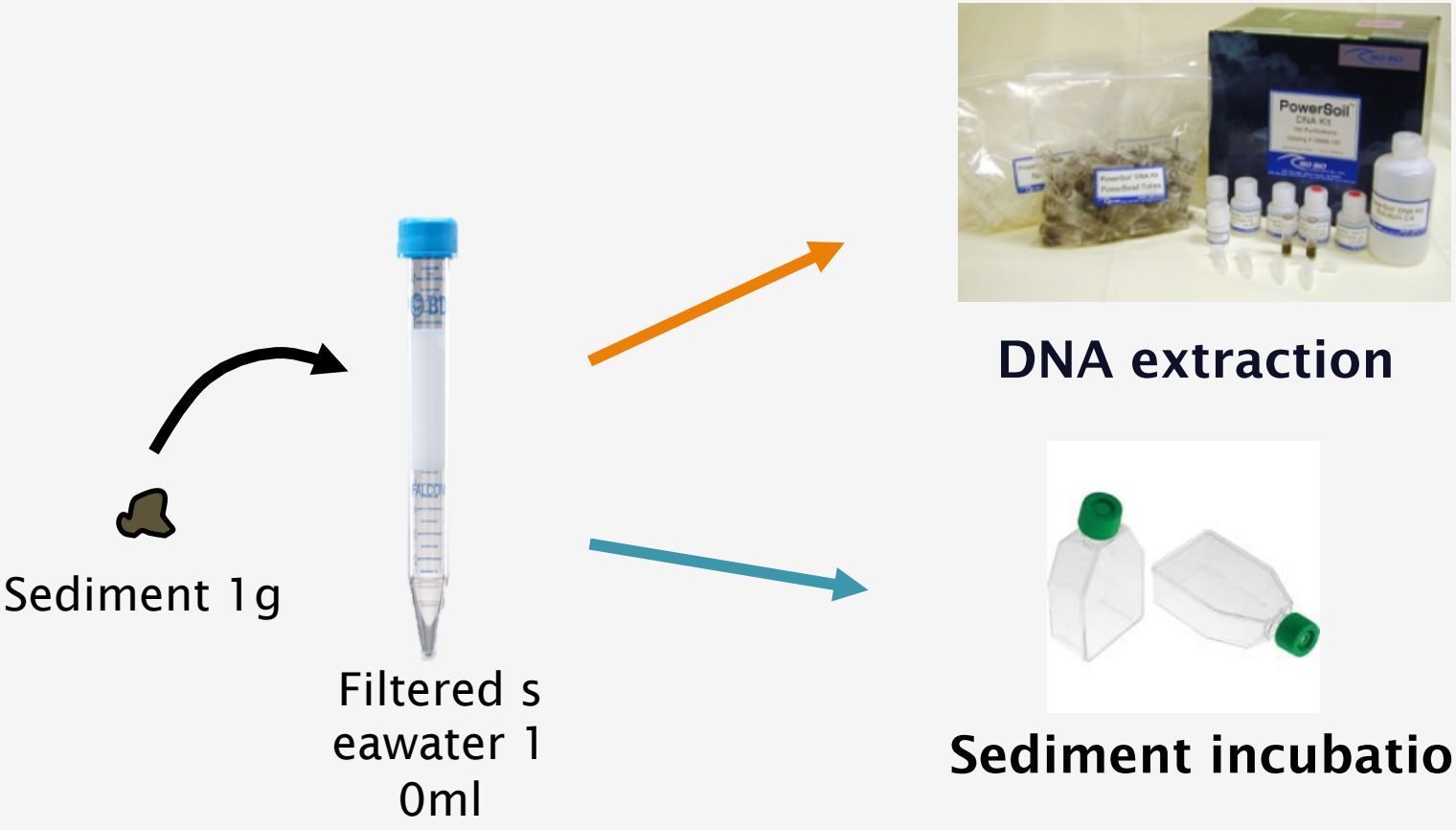
Background

- It could be time, labor, money consuming work that incubation and observation of all sediment samples.
- qPCR assay on sediment samples could be helpful method for pre-screening of *Azadinium* cysts.
- We tried to screening of *Azadinium* cysts using qPCR and selected sediment samples for incubation & cell isolation

Materials & Methods

Sediment treatment

- 1-2 g of sediment was diluted 10 mL of filtered seawater (0.45 μm) to make sediment suspension.



DNA extraction

- We used heating method to remove DNA debris in sedimen t samples which could seriously overestimation effect on re sult of qPCR assay (Kim et al. 2016).

2 mL of sediment susp ension was transferred

Centrifuge & re moving supern atant

1 mL of DW added

Heating and ge ntly mixing

Centrifuge & re moving supern atant

DNA extraction

(PowerSoil DNA Isolation Kit, MoBio, USA)

qPCR assay

- We used Amphidomataceae and *A. poporum*, *A. obesum*, *A. spinosum* specific primer for qPCR assay (Toebe, 2013; Smith, 2015).

Table II: FISH and qPCR primer and probes specific for Azadinium *species*

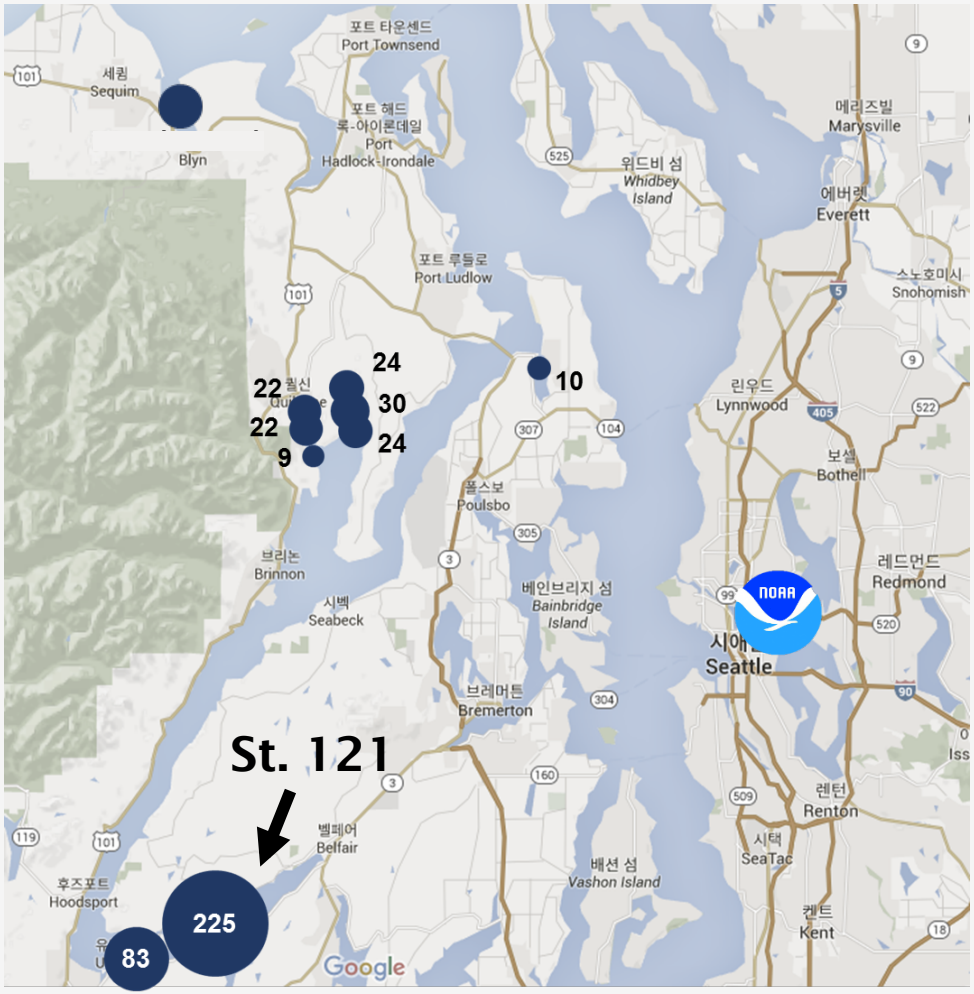
Target species and target gene	Target nucleotide accession numbers	FISH probe (5′–3′)	TaqMan MGB probe (5′–3′)	Primer forward (5′–3′)	Primer reverse (5′–3′)	qPCR amplicon size [number of base pairs (bp)]
A. spinosum, 28S	HQ324896 JN165101 FJ217815	Asp_544 TGG TCG AGT TAC CAG CCC	Aspin77T CGC CCA AAA GGA CTC CT	Asp48F TCG TCT TTG TGT CAG GGA GAT G	Asp120R GGA AAC TCC TGA AGG GCT TGT	72 bp
A. poporum, 28S	HQ324893 HQ324894 HQ324895	Apop_544 CGA GTT ACC AGT TCT CCG	Apop112 TTC CAG ACG ACT CAA A	Apop62F GAT GCT CAA GGT GCC TAG AAA GTC	Apop148R CCT GCG TGT CTG GTT GCA	68 bp
A. obesum, 28S	GQ914936	Aob_544 AAG ACA TTC GAC CTA CCG	Aob163 AAG ACA TTC GAC CTA CCG T	Aob134F AGG GAT CGA TAC ACA AAT GAG TAC TG	Aob208R AAA CTC CAG GGA CAT GGT AGT CTT A	74 bp

Table 1 Sequences of primers and probes used in this study including optimised final concentrations and annealing temperatures for real-time PCR assays

Target	Type	Sequence	Product size	Annealing temperature	Final concentration	Reference
Amphidomataceae						This study
Amp240F	Forward primer	CAA CTT TCA GCG ACG GAT GTC TCG	179 bp	62°C	200 nM	
Amp418R	Reverse primer	AAG CYR CWGGCA TKA GAA GGT AGW GGG			200 nM	

Results

- Amphidomataceae qPCR assay on sediment samples (1/16-18/2016)

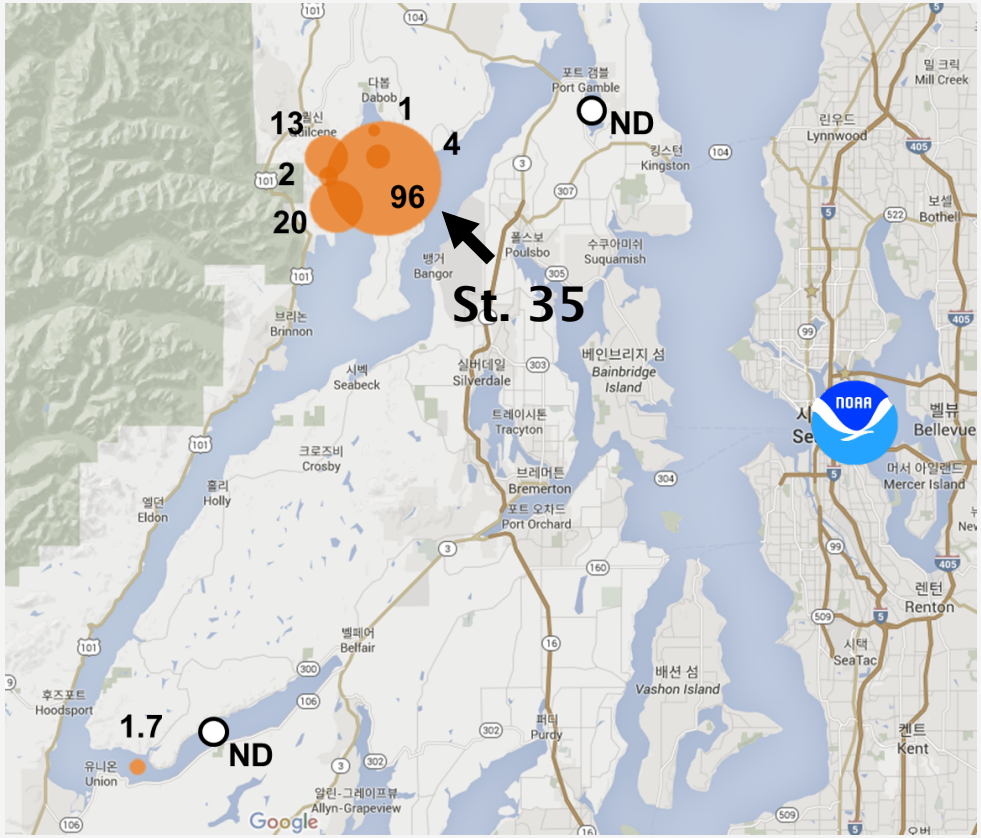


- Sediment DNA was extracted including DNA debris removal step.
- qPCR assay was conducted using Amphidomataceae specific primer (SYBR-Green method).

n Relative abundance of Amphidomataceae cysts

* Cyst abundance value was very roughly calculated based on C_t value of qPCR assay

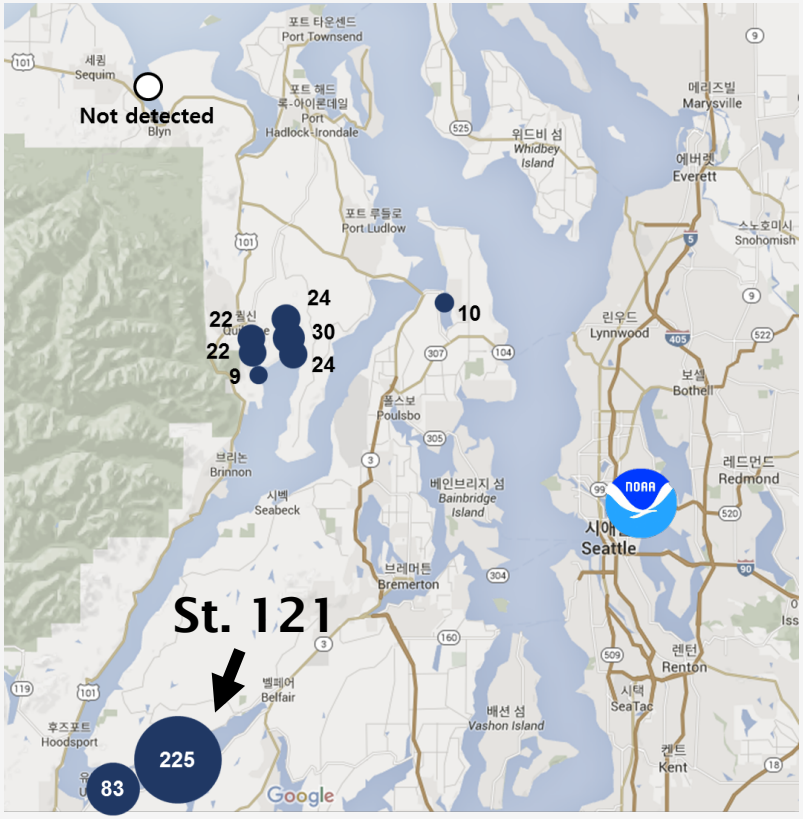
Azadinium specific qPCR assay on sediment samples (1/16-18/2016)

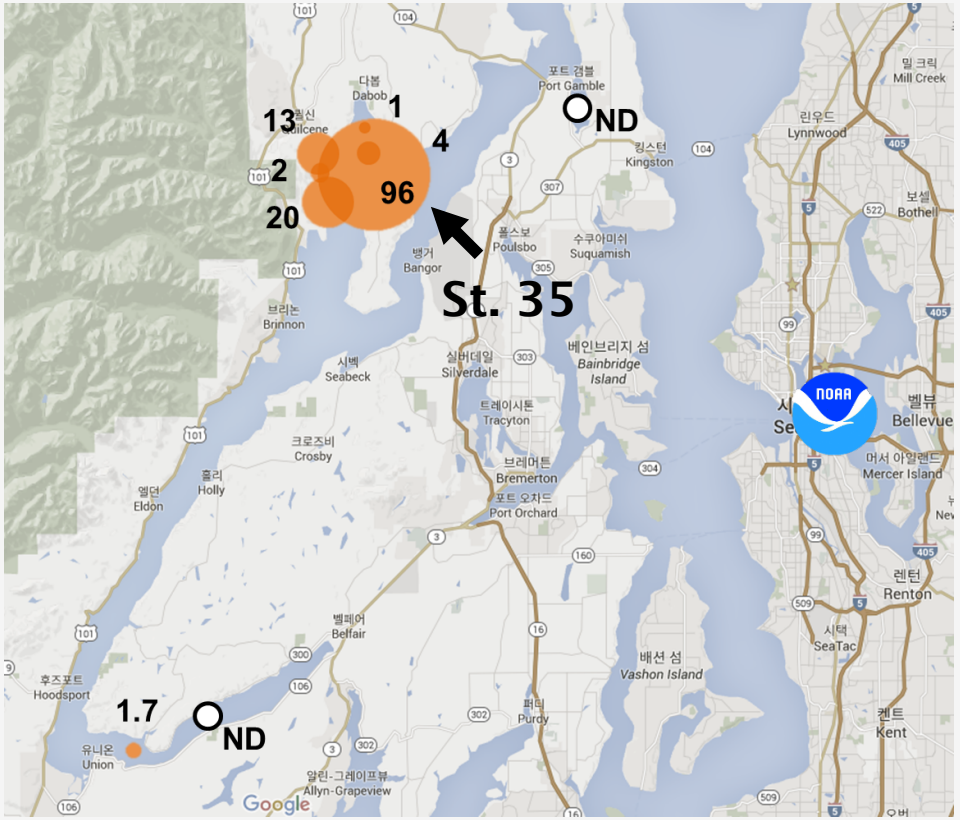


- qPCR assay was conducted using each specific probes of *A. spinosum*, *A. obesum*, *A. poporum*. (Taqman probe method)
- *A. spinosum*, *A. obesum* was not detected in all stations.

Relative abundance of *A. poporum* cysts

* Cyst value was roughly calculated based on C_t value of qPCR assay





Amphidomataceae cysts

A. poporum cysts

- The phenomenon of discordance was observed between c yst distributions of family level and species level.
- This data suggests that other *Azadinium* species could ex ists in Puget Sound area.

Sediment incubation & Isolation

- After qPCR screening, we selected sediment samples f or incubation.
- 1-5 mL of sediment suspension was diluted with 5-50 mL of ESNW-Si medium.
- Incubation in 18˚C, 14:10 (L:D)
- After 5 days, *Azadinium* like cells were isolated.

Possible *Azadinium* (96-2 b3)

Azadinium strains list

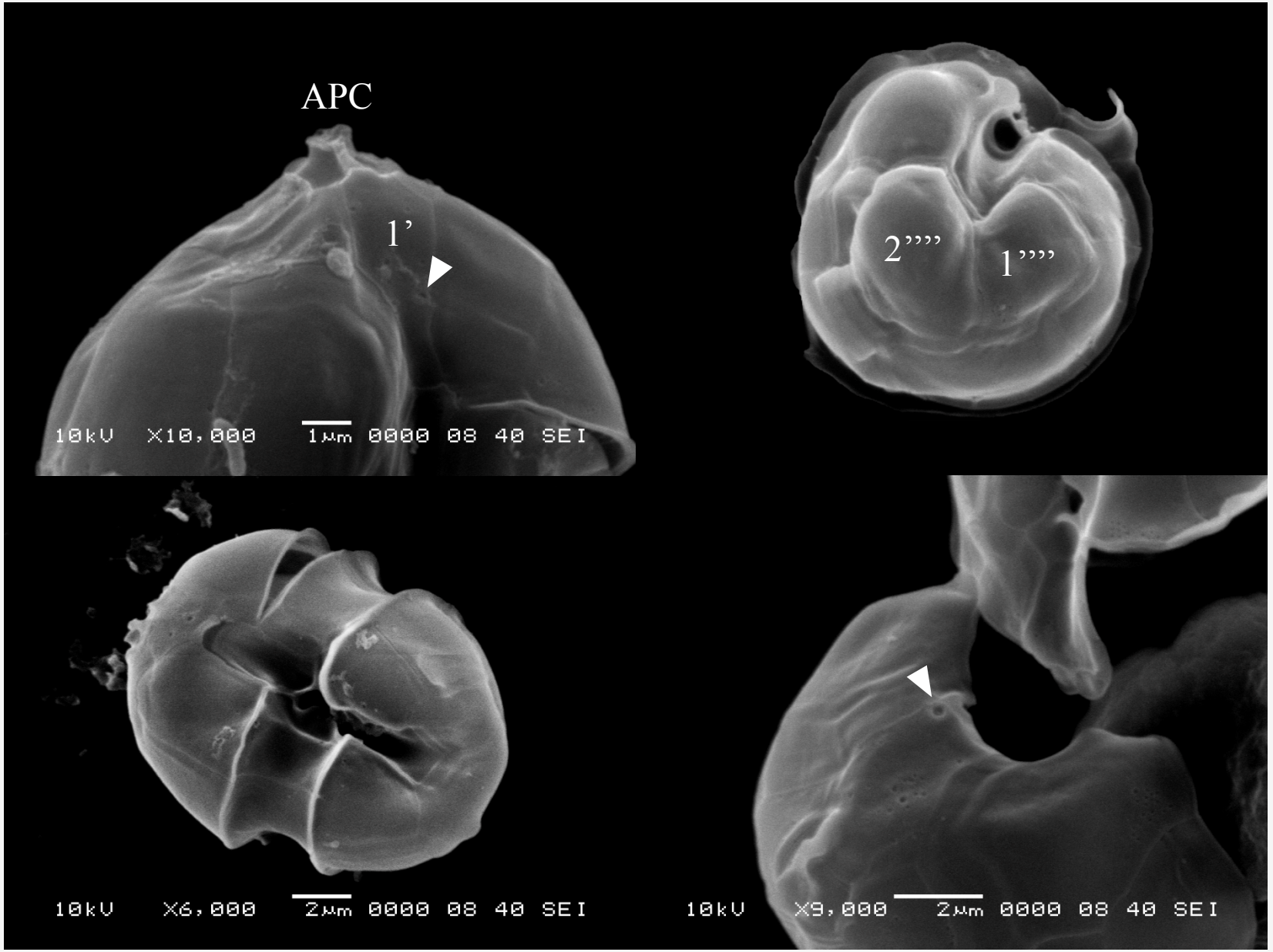
- *Azadinium* has been never successfully isolated in Pacific coastal of North America due to their extr emely low abundance (detected only PCR results).
- Eight possible *Azadinium* strains were isolated.

Species	Strains	Collection date	DNA extraction	RT PCR assay *
Possible *Azadinium*	48-1 b2	2/1/2016	-	-
Possible *Azadinium*	48-1 b3	2/1/2016	-	-
Possible *Azadinium*	48-1 b5	2/1/2016	-	-
Possible *Azadinium*	48-1 f2	2/2/2016	o	o
Possible *Azadinium*	48-1 f8	2/2/2016	o	o
Possible *Azadinium*	96-2 b3	2/2/2016	o	o
Possible *Azadinium*	96-2 b8	2/2/2016	o	o
Possible *Azadinium*	96-1 f4	1/15/2016	-	-

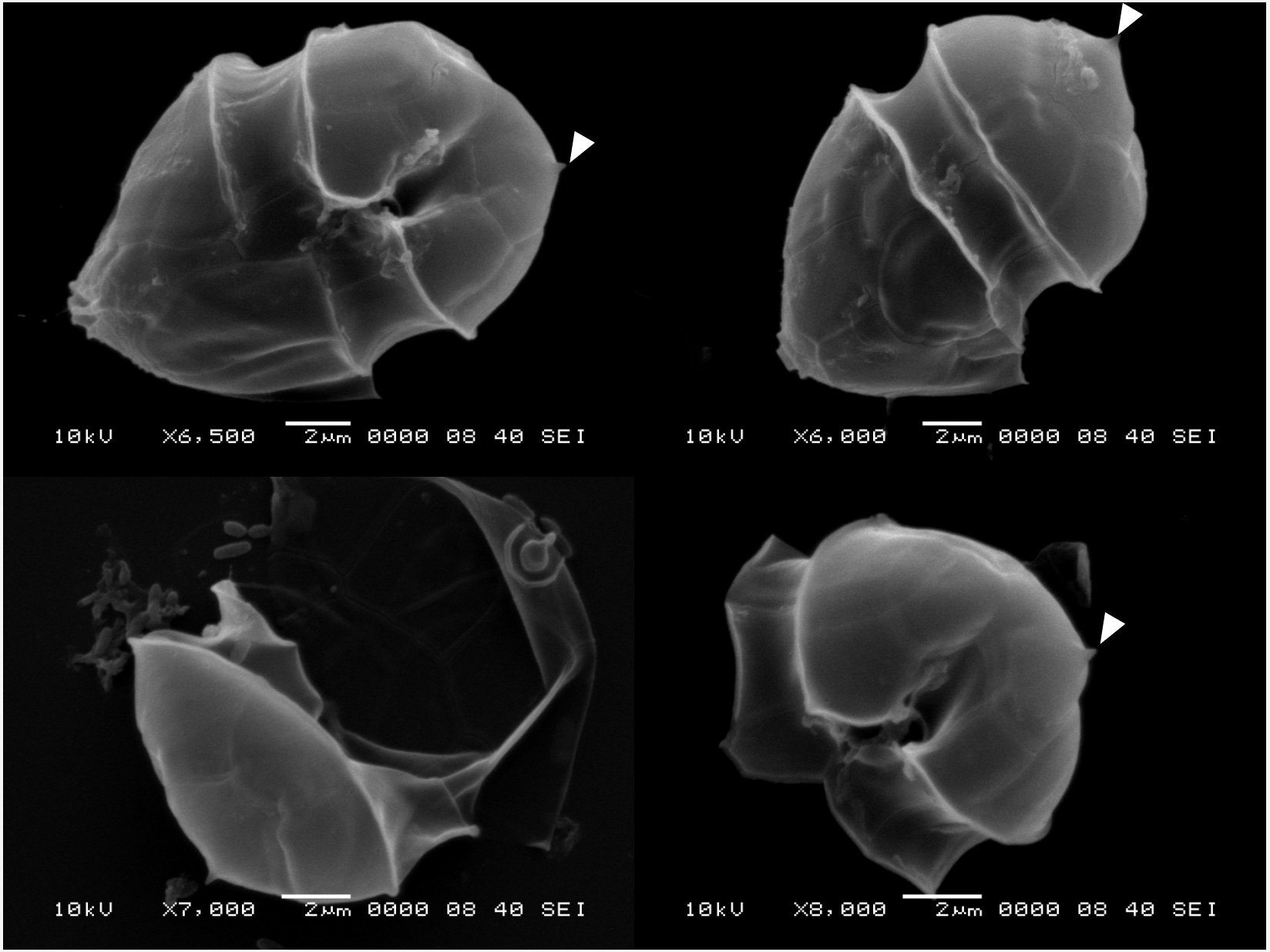
* RT-PCR was conducted using Amphidomataceae specific primer.

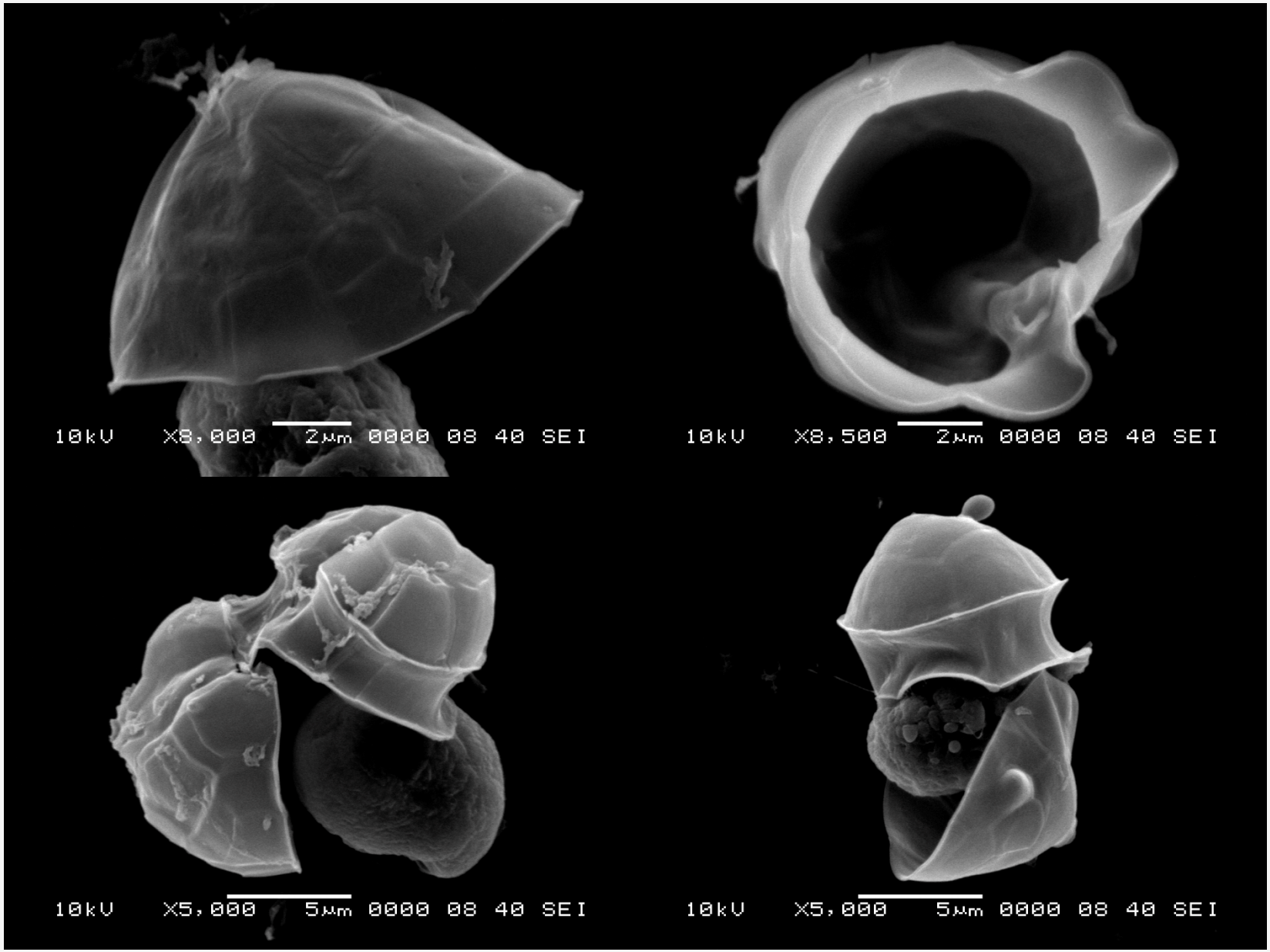
Morphological Characteristics

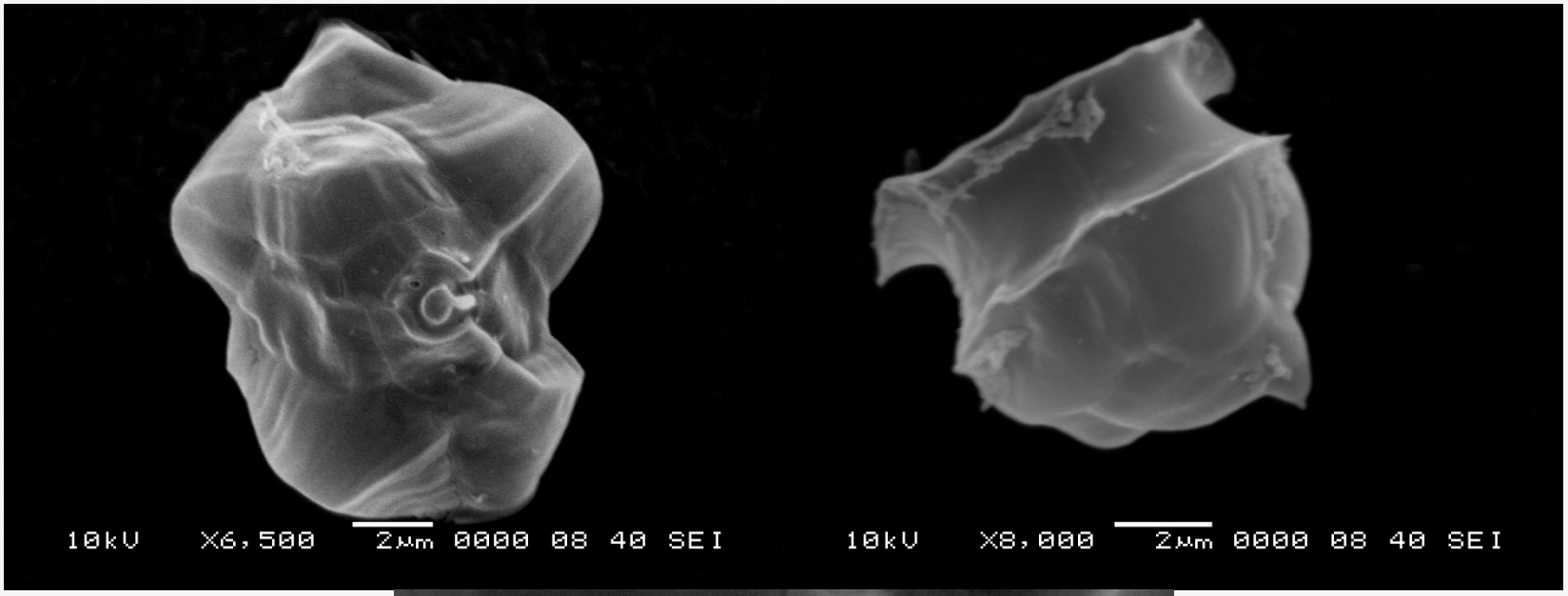
48-1 f2 (*A. obesum*?)

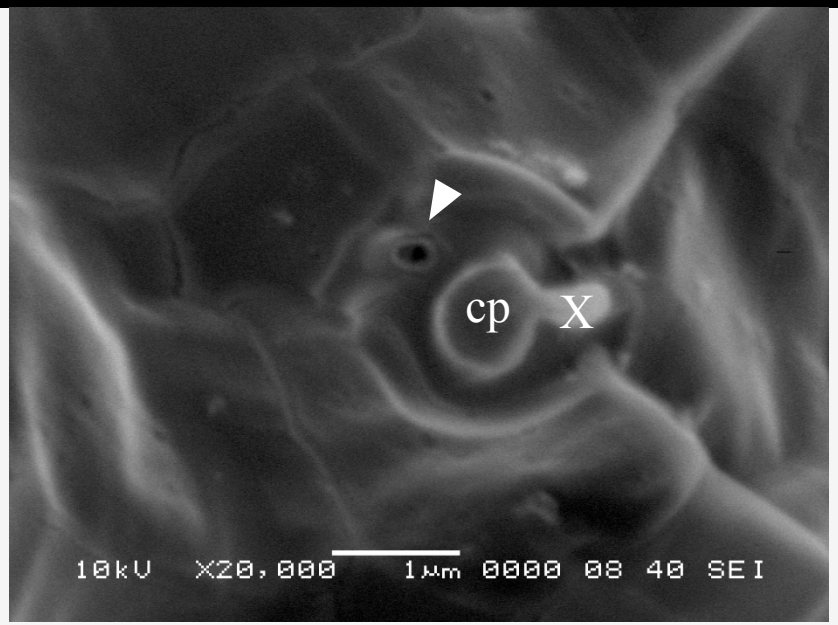


96-2 b3 *A. trinitatum* ?
A. dexteroporum ?




10kV X8,000 2µm 0000 08 40 SEI
10kV X8,500 2µm 0000 08 40 SEI
10kV X5,000 5µm 0000 08 40 SEI
10kV X5,000 5µm 0000 08 40 SEI


10kV X6,500 2µm 0000 08 40 SEI
10kV X8,000 2µm 0000 08 40 SEI


cp
X
10kV X20,000 1µm 0000 08 40 SEI

Thank you for your attention !